Section II: (Remarks)

Claims 1 – 9, 11, and 13 – 19 are pending.

Request for Continued Examination

A Request for Continued Examination under 37 C.F.R. §1.114 is included herewith.

Rejection of Claims and Traversal Thereof

In the March 15, 2010 Final Office Action:

claims 1 – 9, 11, and 13 – 19 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bort et al. (*Biochem. Pharmacol.*, 58(5), 787-796 (1999)) (hereinafter Bort) in view of Gómez-Lechón et al. (*Curr. Drug Metabolism*, 4(4), 292-312 (2003)) (hereinafter Gomez-Lechon)).

These rejections are traversed and reconsideration of the patentability of the pending claims is requested in light of the following remarks.

The Examiner alleges that, upon reading Gomez-Lechon:

> ". . . the ordinary artisan would have realized the advantage of using hepatic cells to study drug metabolism and the limitation of using cells transfected with single CYP; it would have been obvious to the ordinary artisan to try to introduce additional Phase I or Phase II enzymes such that the cell line will reflect the whole multiple CYP enzymes into hepatic cell, while it is clear that single CYP expression has limitation for the intended purpose, would have been obvious because it is within the technical grasp of an ordinary artisan to pursue known options." See, page 7, lines 15 – 22 of the March 15, 2010 Final Office Action.

Applicants respectfully disagree with the Examiner.

As recognized by the Examiner, Gomez-Lechon "do[es] not teach [the] actual practice of the suggested approach of transfecting multiple adenoviral vectors that express[] different phase I or phase II enzymes to cells of hepatic origin." See, page 4, lines 9 to 11 of the March 15, 2010 Final Office Action. Instead, the Examiner alleges that Bort "has demonstrated that this approach is feasible using hepatic cell lines transfected [with a] vector **expressing single CYP enzymes** and assessing hepatic metabolism of diclofenac." See, page 4, lines 21 to page 5, line 1 of the March 15, 2010 Final Office Action (emphasis added). Neither Gomez-Lechon or Bort teach transfecting

multiple adenoviral vectors expressing CYP enzymes to cells of hepatic origin. Instead, the Examiner alleges that "the ordinary artisan having the knowledge of cDNA encoding of Phase I and Phase II enzymes would have reasonable expectation of success to generate adenoviral vectors expressing sense or anti-sense drug metabolizing enzymes to up or down-regulate specific enzymes in a cell of hepatic origin to best mimic the hepatocytes *in vivo*." See, page 5, lines 7 – 11 of the March 15, 2010 Final Office Action.

Applicants respectfully submit that the Examiner is using impermissible hindsight bias to conclude that one of ordinary skill in the art would have been motivated to modify the cell line of Gomez-Lechon so that it expresses additional Phase I or Phase II enzymes. Applicants vigorously disagree that it would have been obvious to one of skill in the art after reviewing Gomez-Lechon and Bort, alone or in combination, to produce the cell lines as disclosed in the instant application.

First, claim 1 in the instant application recites, *inter alia*:

> . . . wherein the expression of said ectopic DNA sequences in the cells transformed with one or more of the aforementioned expression vectors **confers on the transformed cells specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes**, . . ." (emphasis added).

In contrast, Gomez-Lechon and Bort, alone or in combination, do not teach conferring on transformed cells specific phenotypic profiles. The transformed cells of Gomez-Lechon use adenoviral vectors encoding transcription factors to direct the expression of biotransformation enzymes. A single transcription factor can be responsible for the transcriptional activation of several genes. For example, Gomez-Lechon recites that "[u]pon transfection of HepG2 cells with [the transcription factor] C/EBP-alpha, it was found a significant increase in three different CYPs from family 2, suggesting that lack of C/EBO-alpha expression must be relevant in the transcription control of the CRP2 family." See, Gomez-Lechon, page 307, right column, lines 13 – 17. If one of skill in the art started with the Gomez-Lechon cells with the adenoviruses encoding transcription factors, it would be impossible to confer specific phenotypic profiles on transformed cells because a variety of CYP genes could be increased in an unknown manner. Thus, Gomez-Lechon does not teach one of skill in the art how to confer "specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes" on the transformed cells. Bort does not solve this deficiency, alone or in combination, because Bort teaches nothing relating to expressing multiple genes in a transformed cell. As stated by the Examiner, Bort discloses **expressing single CYP enzymes** in transformed cells.

Additionally, for the detailed reasons disclosed in the Declaration under 37 CFR §1.132 of José

Vicente Castell Ripoll (hereinafter the "Declaration"), the method of the instant application is able to confer specific phenotypic profiles on transformed cells compared to the imprecise control of expression of CYP genes through the use of transcription factors as discussed in Gomez-Lechon.

In part one of the Declaration, the transduction of HepG2 hepatoma cells using increasing amounts of adenoviral vectors encoding different CYP proteins (CYP3A4, CYP2B6, and CYP1A2) is shown according to the method of the instant application. The results show that the amount of metabolites generated from each of the enzymes, a direct measure of the corresponding enzymatic activity of the different CYP enzymes, increased linearly as a function of the MOI of the adenoviruses used for transduction. Thus, the method of the instant application allows individual control of the enzymatic activities of the biotransformation enzymes. For example, in Figure 1 a specific phenotypic profile is conferred on transformed cells to approximate a human hepatocyte culture; compare Figure 1A (human hepatocytes) to Figure 1F (transformed cell). In contrast, when transcription factors are used as in Gomez-Lechon, an unknown number of enzymes are increased in an unknown manner. The specific control of expression resulting in specific phenotypic profiles is not possible using the transcription factors of Gomez-Lechon. Therefore, it would not be obvious to one of skill in the art, reading Gomez-Lechon, to modify the cells to express genes encoding ectopic genes so that specific phenotypic profiles of Phase I or Phase II drug biotransformation enzymes are conferred on transformed cells. Bort, either alone or in combination with Gomez-Lechon, does not cure this deficiency because it does not discuss modifying cells with a plurality of adenoviral expression vectors.

Further, part two of the Declaration describes a comparative example with respect to Gomez-Lechon wherein the expression of CYP3A4 in HepG2 cells is compared using the techniques of Gomez-Lechon and the instant application. The transformed cells of the instant application are able to approximate human hepatocytes whereas the cells of Gomez-Lechon are not. As can be seen in Figure 3, the cells constructed using the method of the instant application (Adv-CYP3A4) approximate the *in vivo* activities of human hepatocytes. By comparison, three cells constructed with the transcription factors of Gomez-Lechon express significantly less enzyme, up to 1000-fold less, than that observed in human hepatocytes. Consequently, infection with an adenovirus encoding key transcription factors alters the expression (mRNA), but not the function of hepatic drug-metabolizing enzymes. Hence, the cells of Gomez-Lechon are not suitable for the instant application and would not lead one of skill in the art to modify them so that "specific phenotypic profiles" could be conferred on the transformed cells, as recited in claim 1 of the instant application. Bort, alone or in combination with Gomez-Lechon, does not make up this deficiency because it does not motivate, teach, or suggest a method to confer specific phenotypic profiles on transformed cells to one of skill in the art.

Second, the Examiner stated: "it would have been obvious to the ordinary artisan to try to introduce additional Phase I or Phase II enzymes such that the cell line will reflect the whole multiple CYP enzymes into hepatic cell" and concluded that "[i]f it leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense." See, page 7, line 17 to page 8, line 11 of the March 15, 2010 Final Office Action. Applicants respectfully disagree. MPEP §2145X(B) states that obvious to try may support a conclusion of obviousness when one skilled in the art is choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. That is not the case here.

As disclosed in Gomez-Lechon, there are many different methods of increasing expression of CYP enzymes in hematoma cells, not a finite number of identified, predictable solutions. For example, Gomez-Lechon discusses using (1) adenoviral vectors encoding transcription factors, (2) adenoviral vectors encoding transcription repressors, (3) DNA methylation/histone deacetylation, (4) actyltransferase co-activators, and (5) transcriptional co-activators. In addition, Bort discusses (6) inserting cDNA's using blunt-ended cloning to increase expression of CYP enzymes. These six different methods of increasing expression of CYP enzymes are discussed in the cited references and still none of them are appropriate for conferring on transformed cells specific phenotypic profiles. This is not the "finite number of identified, predictable solutions" discussed in MPEP §2145X(B).

Also, these methods are not predictable. As stated previously, a transcription factor can increase expression of multiple enzymes. In other cases, multiple transcription factors are necessary to increase expression of a single enzyme. Still further, the increase in mRNA because of a transcription factor does not mean that there will be an increase in protein activity, in particular for enzymes involved in biotransformation. As is known to those skilled in the art, for some CYPs there is no correlation between the mRNA level and the protein activity level.[1] mRNA contents cannot be used as a surrogate for enzymatic activity until the relationship between mRNA and protein activity has been established. Therefore, the use of adenoviral vectors to control the activity of the corresponding biotransformation enzymes was unexpected over Gomez-Lechon because Gomez-Lechon measured mRNA levels in the cell not the enzyme products.

Bort does not cure these deficiencies because it is solely concerned with the identification of the biotransformation enzyme responsible for the metabolism of diclofenac. In order to do that, Bort prepares different populations of epithelial cells that have been genetically modified so that they each express a single human CYP to find out the best CYP for metabolizing diclofenac. Bort does not

---

[1] See, e.g., Rodriguez-Antona *et al.* (2001). Cytochrome P-450 mRNA Expression in Human Liver and its Relationship with Enzyme Activity. *Arch. Biochem. Biophys*, 393:308-315. See, Appendix A.

mention that a given compound can be metabolized using a plurality of biotransformation enzymes, let alone that biotransformation enzyme might be simultaneously expressed in the cell in an individually controlled fashion. Thus, a person skilled in the art would not have arrived to the method having the features of claim 1 by considering the teaching of Gomez-Lechon or Bort, either singly or in combination.

Claims 2 – 9, 11, and 13 – 19 are dependent on claim 1 and are therefore patentable over Bort in light of Gomez-Lechon for the same reasons as claim 1.

Reconsideration and withdrawal of the rejection of claims 1 – 9, 11, and 13 – 19 under 35 U.S.C. 103(a) over Bort in view of Gomez-Lechon is respectfully requested.

Petition for Extension of Time/Fees Payable

Applicants hereby petition for a two (2) month extension of time, extending the deadline for responding to the March 15, 2010 Final Office Action from June 15, 2010 to August 16, 2010. August 15, 2010 is a Sunday. As such, the fee of $245.00 specified in 37 CFR §1.17(a)(2) for a small entity for such two (2) month extension is due.

Applicants herewith submit a Request for Continued Examination. As such, the fee of $405.00 specified in 37 C.F.R. §1.17(e) for a small entity for such RCE is due.

The total fee of $650.00 is being paid using Electronic Funds Transmission. Authorization is also hereby given to charge any deficiency in applicable fees, or credit any overcharges, for this response to Deposit Account No. 13-4365 of Moore & Van Allen PLLC.

Conclusion

Based on the foregoing, claims 1 – 9, 11, and 13 – 19 are in form and condition for allowance. If any additional issues remain, the Examiner is requested to contact the undersigned attorney at (919) 286.8000 to discuss same.

Respectfully submitted,

MOORE & VAN ALLEN PLLC

Date: July 27, 2010

By: _____
Tristan A. Fuierer
Registration No. 52,926
Moore & Van Allen PLLC
430 Davis Drive, Suite 500
Morrisville, NC 27560-6832
Telephone: (919) 286-8000
Facsimile: (919) 286-8199

Date: July 27, 2010

By: _____
Andrew D. Gerschutz
Registration No. 65,529
Moore & Van Allen PLLC
430 Davis Drive, Suite 500
Morrisville, NC 27560-6832
Telephone: (919) 286-8000
Facsimile: (919) 416-8365

ELECTRONICALLY FILED USING THE EFS-WEB ELECTRONIC FILING SYSTEM OF THE UNITED STATES PATENT & TRADEMARK OFFICE ON JULY 27, 2010